15 JAN 1999

FORM PTO 1390
(REV 5-93) US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371

ATTORNEY DOCKET NUMBER
1208/P502PCTUS

U.S. APPLICATION NO.
(if known, see 37 CFR 1.5)
NEW 09/214848

International Application No.	International Filing Date	Priority Date Claimed
PCT/JP97/02438	July 14, 1997	July 15, 1996

Title of Invention
REMEDIES/PREVENTIVES FOR VIRAL INFECTIONS, PROCESS FOR PREPARING THE SAME, AND METHOD FOR PREVENTING/TREATING VIRAL INFECTIONS

Applicant(s) For DO/EO/US
Teruaki SEKINE

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.

2. [] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.

3. [] This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).

4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   a. [] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [X] A translation of the International Application into English (35 U.S.C. §371(c)(2)). **ATTACHMENT A**

7. [] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3)).
   a. [] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] have been transmitted by the International Bureau.
   c. [] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [] have not been made and will not be made.

8. [X] An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)). **ATTACHMENT B**

9. [] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 10. to 13. below concern other document(s) or information included:

10. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98. **ATTACHMENT C**

11. [] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

12. [] A FIRST preliminary amendment.

    [] A SECOND or SUBSEQUENT preliminary amendment.

13. [X] Other items or information: SMALL ENTITY DECLARATION

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) NEW	INTERNATIONAL APPLICATION NO. PCT/JP97/02438	ATTORNEY'S DOCKET NO. 1208/P502PCTUS

14. [X] The following fees are submitted	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
[X] Search Report has been prepared by the EPO or JPO..... $ 840.00		
[] Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... $ 970.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	-20 =		X $18.00	$	
Independent Claims	- 3 =		X $78.00	$	
Multiple dependent claim(s) (if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$840.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28)				$420.00	
SUBTOTAL =				$420.00	
Processing fee of $130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$420.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40 per property +				$	
TOTAL FEES ENCLOSED =				$420.00	
				Amount to be refunded	$
				Amount to be charged	$

a. [X] A check in the amount of **$420.00** to cover the above fees is enclosed. A duplicate copy of this form is enclosed

b. [] Please charge my Deposit Account No. 23-0975 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-0975. A duplicate copy of this form is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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33,367
REGISTRATION NUMBER

January 14, 1999

[CHECK NO. 31315]
[99-0024*/WMC/1208]

Applicant or Patentee: _____
Serial or Patent No.: _____ Atty. Dkt. No.: _____
Filed or Issued: _____
For: REMEDIES/PREVENTIVES FOR VIRAL INFECTIONS, PROCESS FOR PREPARING THE SAME, AND METHOD FOR PREVENTING/TREATING VIRAL INFECTIONS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled REMEDIES/PREVENTIVES FOR VIRAL INFECTIONS, PROCESS FOR PREPARING THE SAME, AND METHOD FOR PREVENTING/TREATING VIRAL INFECTIONS described in

(X) the specification filed herewith
( ) application serial no. _____, filed _____
( ) patent no. _____, issued _____.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

(X) no such person, concern, or organization
( ) persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27)

FULL NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
TERUAKI SEKINE		
Signature of Inventor: Teruaki Sekine	Signature of Inventor	Signature of Inventor
Date: January 6, 1999	Date	Date

SPECIFICATION

REMEDIES/PREVENTIVES FOR VIRAL INFECTIONS, PROCESS FOR PREPARING THE SAME, AND METHOD FOR PREVENTING/TREATING VIRAL INFECTIONS

TECHNICAL FIELD

This invention relates to remedies or preventives for viral infections containing, as the main ingredient, autologous lymphocytes cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase, which can be used for not only preventing or treating the viral infections so as to improve the therapeutic or preventive effects on the viral infections, but also sufficiently preventing immunodeficiency or immunosuppression and postoperative or post-transplantation viral infections, and further relates to a method for preparing the aforementioned remedies or preventives and a therapeutic method for preventing or treating the viral infections by use of the aforementioned remedies or preventives.

BACKGROUND ART

Living bodies are protected from various infections and cancers by their own immunity system. In general, humoral immunity and cellular immunity have been known as the immunity system. Complements being one kind of serum proteins, lysozyme and antibody bestow the humoral immunity, and macrophage and cells such as NK cells and T cells bestow the cellular immunity. The cellular immunity brought about mainly by the NK cells and T cells seems to be importance to the immune reaction to cancers.

Cells are sorted according to their surface markers appearing on the

surfaces thereof. On the mature T cell, CD3 emerges as the surface marker. Further, CD8 appears on a killer T cell having antitumor activity and antiviral activity, and CD4 appears on a helper T cell which are closely related to the activation of cells having various immune systems. Besides, the mature T cells bear T cell receptors which have high affinity relative to specific antigens. The T cells bear only a single type of T cell receptors through which antigen-specific T cells are activated. Furthermore, different sorts of T cell receptors which are very diverse exit within a living body.

Incidentally, the complements which singularly inactivate viruses, lysozymes, and macrophages which eat greedily the complements are important to protection against viral infections at the first stage. From a few days after getting a viral infection, viral-specific antibodies are rapidly increased, and consequently, fulfill the important function to protect against the viral infections. As the viral-specific antibody, there have been known a neutralizing antibody serving to inactivate the viruses and a specific antibody capable of exerting ADCC activity having a function of specifically removing cells infected with viruses with the cooperation of T cells and so forth, though the ADCC activity per se cannot make viruses inactive. Therefore, the viral-infection protective reaction takes place principally by the humoral immunity and cellular immunity. Thus, the defensive reaction of the living body against the cancer is remarkably different from that against the viral infections, although both the reactions occur through the immunity system.

The virus which is essentially extraneous relative to the living body has the nature of living with its host cell to proliferate. From one infective virus, thousands to tens of thousands of virus particulars are brought forth at once with explosive speed. However, the cancer naturally brought about due to

mutation of host-derived cells requires twenty hours or thereabout at the earliest to cause binary fission. Of the various viruses, there are viruses lurking in infected cells of the living body, with the result of which the living body is latently infected therewith. As the latently infective viruses, there have been known various herpesviruses and retroviruses. These viruses frequently evolve to pathogenic viruses when the immunity power of the host is weakened.

As noted above, the origins of the cancer and affected with a viral infection are wholly different in living body defensive mechanism and proliferating speed and mechanism. Accordingly, the therapy in the treatment for cancer is not necessarily effective in the preventive or therapeutic treatment for viral infections. In actual fact, an anticancer drug clinically used differs from an antiviral agent in most cases. Antitumor activation by stimulation of lymphocytes, particularly, T cells and NK cells, of a cancer patient by interleukin-2 was reported by Rosenberg (New Eng. J. Med., 319, 1678(1988)). The inventors of the present invention have already disclosed a stimulation method for proliferation of lymphocytes, by which the lymphocytes of the cancer patient are stimulated with a coagulated anti-CD3 antibody and interleukin-2, consequently to be proliferated (Japanese Patent Application Public Disclosure No. 03-80076(A)).

A remedy for cancer by use of the aforesaid lymphocytes has been reported (Human Cell, 7, pp.121-123(1994). Further, Kliona M. Rooney et al. have reported that lymphocytes derived from a donor of the bone marrow, which are proliferated by stimulation with the interleukin-2 and Epstein-Barr virus transformation lymphocytes, are effective in proliferating the lymphocytes participating with Epstein-Barr viruses brought forth in bone marrow transplantation (Lancet, 345, 9(1995)). Also, Elizabeth A. Walter et al. have

reported that lymphocytes derived from a donor of the bone marrow, which are proliferated by stimulation with the anti-CD3 antibody, fibroblast infected with cytomegalovirus and interleukin-2 are effective in remedying cytomegalovirus infections in bone marrow transplantation (New Eng. J. Med., 333(16), 1038(1995)). However, a remedy for viral infections by use of autologous lymphocytes of a patient, which are proliferated by being stimulated with the coagulated anti-CD3 antibody and interleukin-2 has not at all been reported so far. Scott et al. carried on the research concerning a remedy of HIV by using lymphocytes showing peculiarity relative to HIV, which are prepared from the lymphocytes derived from the patient by use of PHA, interleukin-2, feeder cells and OKT-3, but has not succeeded in remedying HIV infections by that remedy (Nature Medicine, 1(4), pp. 330-336 (1995)).

However, Japanese Patent Appln. Disclosure No. 03-80076(A) is concerned with the remedy for cancer by use of the lymphocytes which are proliferated by being stimulated with the CD3 antibody and interleukin-2, but does not reveal whether or not the lymphocytes proliferated by being stimulated with the CD3 antibody and interleukin-2 are efficacious against infectious cancers.

Likewise, Human Cell merely discloses the remedy for cancer by use of the lymphocytes proliferated by being stimulated with the CD3 antibody and interleukin-2, but does not elucidate that the lymphocytes proliferated by being stimulated with the CD3 antibody and interleukin-2 are efficacious against infectious cancers.

Although Kliona M. Rooney et al. reported that the lymphocytes derived from the donor of the bone marrow proliferated by stimulation with the virus transformation lymphocytes are effective in proliferating the lymphocytes

participating with Epstein-Barr viruses brought forth in bone marrow transplantation, the lymphocytes used therefor were not derived from the patient, but obtained from the donor of the bone marrow and that of health, who does not contract any infection. That is, it is not evident from the report that the lymphocytes, which are obtained by proliferating with stimulation the lymphocytes derived from the patient contracting the infections and showing the symptoms of the infections, are efficacious against the infections. Moreover, use of an anti-CD3 antibody which coagulates in the process of stimulation to proliferate the lymphocytes is not confirmed in the report, and in conclusion, the method for stimulating and proliferating the lymphocytes as proposed in the report is fundamentally distinct from that using autologous lymphocytes.

Also, the lymphocytes used in the report of Elizabeth A. Walter, disclosing that lymphocytes derived from a donor of the bone marrow proliferated by being stimulated with the anti-CD3 antibody, fibroblast infected with cytomegalovirus and interleukin-2 are effective in remedying cytomegalovirus infections in bone marrow transplantation, were not derived from the patient, but obtained from the donor of the bone marrow and that of health, who does not contract any infection. That is, it is not evident from the report that the lymphocytes, which are obtained by proliferating with stimulation the lymphocytes derived from the patient contracting the infection and showing the symptoms of the infections, are effective against the infections. Moreover, use of an anti-CD3 antibody which coagulates in the process of stimulation to proliferate the lymphocytes is not confirmed in the report, and consequently, the method for stimulating and proliferating the lymphocytes as proposed in this report is fundamentally distinct from that using autologous lymphocytes. Therefore, it can be said that the remedy proposed in this report is unsuitable

for a patient contracting a viral infection.

An object of the present invention is to provide remedies or preventives for the treatment or prevention of viral infections, which can more improve the effects of treatment and prevention of viral infections, effectively prevent postoperative or post-transplantation viral infections, and remarkably increase the efficiency of proliferating lymphocytes.

Another object of the invention is to provide, in the concrete, remedies or preventives for the treatment or prevention of viral infections, which contain, as the main ingredient, autologous lymphocytes proliferated by being cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase.

Still another object of the invention is to provide remedies or preventives for viral infections, which are markedly efficacious for viral infections in patients with immunodeficiency or immunosuppression.

Yet another object of the invention is to provide a method for preparing remedies or preventives for viral infections, which contain, as the main ingredient, autologous lymphocytes cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase.

A further object of the invention is to provide a therapeutic method for preventing or treating the viral infections by use of remedies or preventives for viral infections, which contain, as the main ingredient, autologous lymphocytes cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase.

Lymphocytes collected from a patient contracting a viral infection are cultivated in a culture medium made into a solid phase, which contains an anti-CD3 antibody and interleukin-2 so as to be proliferated immediately after

the cultivation. After the lapse of about two weeks, the number of lymphocytes becomes about 1000 to 10000 times.

DISCLOSURE OF THE INVENTION

The remedies or preventives for viral infections according to the present invention are featured in that the aforementioned autologous lymphocytes are proliferated by being cultivated in the culture medium containing the anti-CD3 antibody and interleukin-2 made into a solid phase.

THE BEST MODE FOR CARRYING OUT THE INVENTION

The remedies or preventives for viral infections according to the present invention contains, as the main ingredient, autologous lymphocytes cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase. The viral infections specified herein are defined as a state in which a patient has already contracted a viral infection in treatment or prevention therefor or an immunosuppression state in which a patient is exposed to the possibility of contracting the viral infection.

Generally, cells are sorted according to their surface markers appearing on the surfaces thereof. On the mature T cell, CD3 emerges as the surface marker. Further, CD8 appears on a killer T cell having antitumor activity and antiviral activity, and CD4 appears on a helper T cell, which are closely related to the activation of cells having various immune systems. Besides, the mature T cells bear T cell receptors which have high affinity relative to specific antigens. The T cells bear only a single type of T cell receptors through which antigen-specific T cells are activated. Furthermore, different sorts of T cell receptors which are very diverse exit within a living body.

On the other hand, as a viral infection, there are enumerated infections caused by viruses belonging to various herpesvirus groups, which are typified

by cytomegalovirus, Esstein-Barr virus, herpes simplex virus, and varicellazoster virus, viruses belonging to various retrovirus groups, which are typified by human immunodeficiency virus and HTLV, and various hepatitis viruses typified by hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, and infections due to causal bacteria such as influenza viruses. The remedies or preventives for viral infections according to this invention are especially efficacious against the viral infections caused by the viruses of the herpesvirus groups, but the application of the present invention is by no means limited to the viruses enumerated.

In particular, the present invention is effective in remedying patients with immunodeficiency or immunosuppression. The immunodeficiency belongs to either a congenital immunodeficiency syndrome or an acquired immunodeficiency syndrome. The congenital immunodeficiency is typified by the Wiskott-Aldrich syndrome, and the acquired immunodeficiency is typified by AIDS and ARC. The immunosuppression is caused in the state of giving immunosuppressing remedial agents for organ transplantation or bone marrow transplantation, bearing tumor, getting scaled, following a surgical operation, or producing side effects on immune cells due to medication of remedial agents. The causes for immunodeficiency or immunosuppression are not limited thereto. The remedies or preventives according to this invention are especially effective for patients medically deemed to contract any type of immunodeficiency or immunosuppression.

The desired lymphocytes of the patient can be gathered from peripheral blood, lymph nodes, or the thymus of the patient. Any autologous lymphocytes gathered from any tissues or organs of the patient may be efficiently used.

Particularly, the lymphocytes of the peripheral blood from the veins of the patient are serviceable, because the blood can easily be drawn therefrom without imposing a burden on the patient. For the purpose of preventing the peripheral blood from coagulating, there may be added heparin or citric acid thereto, but this measure is not absolutely necessary to this invention. In this embodiment, separation of the desired lymphocytes from the peripheral blood is carried out by a density centrifugation method. The density centrifugation is fulfilled by use of Ficoll-Hypaque, monopoly resolution or the like, but these separation methods should not be understood as being limitative.

The blood may be gathered at a time on the order of 0.1 to 500 ml. It is desirable to gather 1 to 100 ml of blood, and more preferably 10 to 50 ml. The method according to this invention can be performed by use of a large amount of blood or even a small amount of blood. In the case of drawing a little bit of blood, a burden on the patient can be reduced to a minimum. Thus, the blood of the order of 50 ml may preferably be gathered at a time. From blood gathered at a time, desired cells available for several times of administering can be prepared, but may preferably be prepared by gathering the blood little by little several times in accordance with the number or interval of administering.

Any antibody capable of recognizing CD3 may be used as the anti-CD3 antibody. As one example, OKT3 antibody (made by Ortho Pharmaceutical Corp.) can be used. As a supporter for making the anti-CD3 antibody into a solid phase, various types of flasks, culture dishes, plates and bags may be used. A solid-phase method for antibody can be performed by non-specific adsorption or chemical bonding. However, the solid-phase method in this invention should not be limited thereto. Any solid-phase method capable of

stimulating lymphocytes with the anti-CD3 antibody may be adopted. Further, any type of interleukin-2 may be used insofar as it can activate lymphocytes, particularly, T cells. The cultivation of cells in in-vitro can be effected by use of RPMI 1640 culture medium, DMEM culture medium, or non-serum culture medium. To the culture medium, there may be added blood serum, protein, amino acid, saccharide and/or antibiotic to cultivate the lymphocytes.

The lymphocytes cultivated in the in-vitro may be suspended in a buffer solution such as physiological saline and phosphate buffer solution to be administered to the patient. The lymphocytes having the cell concentration in the range of 1×10^4 parts/lit. to 1×10^8 parts/lit. may be used. It is preferable to use the lymphocytes having the cell concentration in the range of 1×10^6 parts/lit. to 1×10^8 parts/lit. It is desirable to add protein and so on to the cell-suspended solution to prevent the cells from coagulating and adhering to a receptacle. As the protein applied thereto, human albumin may be used in concentrations of from 0.1 w/v% to 10 w/v%, for instance.

Although the remedies/preventives of the invention may be administered to the patient through any administering route such as the abdominal cavity, artery, muscle and subcutaneous tissue, it is recommended to administer the remedies/preventives through the vein for the convenience of surgery. The administration of the remedies/preventives may be carried out when or before showing the infections. In this embodiment, the administration of the remedies/preventives is effected when showing the infections, since the curative effect of the remedies of the present invention can clearly be confirmed at that time. It would be surmised by a person skilled in the field that the agents having the curative effect have the preventive effect on the infections as a matter of course. Thus, the remedies/preventives according to the present

invention can be used for not only remedying the infections, but also preventing the infections.

For giving the remedies or preventives to the patient, the lymphocytes proliferated by being stimulated with the anti-CD3 antibody made into a solid phase and interleukin-2 or the lymphocytes expansively cultivated in a suitable culture medium after providing stimulation thereto may be used. To the culture medium for expansively cultivating the lymphocytes, the interleukin-2 may be added according to need. Although the lymphocytes gathered in this invention are cultivated in the culture medium containing the anti-CD3 antibody made into a solid phase and interleukin-2 so as to be proliferated, various cytokines or proliferating factors may be added thereto, or the lymphocytes may be stimulated with a specific antigen to be proliferated.

Some clinical embodiments to which this invention was applied will be described hereinafter, but this invention does not contemplate imposing any limitation on the conditions of these examples.

[EMBODIMENT 1] PREPARATION OF A FLASK FOR MAKING OKT3 ANTIBODY INTO A SOLID PHASE

To a cultivating flask (made by Sumitomo Bakelite Co., Ltd.) having a bottom area of 225 cm^2, 30 ml of PBS(-) solution containing OKT3 antibody having a concentration of 5 μ g/ml was added. The flask having its bottom soaked in the solution was left for 2 hours at room temperature. Thus, an OKT3-solidifying flask for making OKT3 antibody into a solid phase was prepared and retained for use at 4 ℃.

[EMBODIMENT 2] PREPARATION OF LYMPHOCYTES

From the vein of a patient contracting the Wiskott-Aldrich syndrome being the herpes simplex infections and EB viral infections, 50 ml of blood was

drawn as heparin-added peripheral blood. To the blood thus dranw, RPMI 1640 culture medium of the same quantity as the blood was added. The blood with the culture medium was superposed on Lymphosepar-I (made by Immuno Biological Laboratory) distributed into several centrifugal beakers each having a capacity of 15 ml, and then, exposed to centrifugal force at 1800rpm for 15 minutes. Thereafter, the lymphocytes in the layered state were gathered with a pipette and mingled with RPMI 1640 culture medium, and then, subjected to centrifugal separation (at 1800 rpm for 10 minutes). Upon removing the top layer of each beaker, cell pellets thus obtained were thoroughly loosened. And then, to the cell pellets, there was added 50 ml of culture solution (obtained by adding 10% of human serum and 700 U/ml of interleukin-2 to the RPMI 1640 culture medium containing 60 μ g/ml of kanamycin, 20 μ g/ml of streptomycin, 2 mM of glutamine, 1 mM of oxaloacetic acid, 1 mM of pyruvate acid sodium, 10 mM of HEPES, and 0.2 U/ml of insulin), thereby to prepare a cell-suspended solution.

The OKT3-solidifying flask prepared in Embodiment 1 was washed with PBS(-) two times. After washing, the aforementioned cell-suspended solution was inoculated into the OKT3-solidifying flask and cultivated in the atmosphere of 5% of carbonic acid gas in saturated humidity at 37 ℃. After 3 days, 50 ml of culture solution was added thereto, and further, after 4 days, 150 ml of culture solution was added. Upon suspending the culture solution, 125 ml of culture solution in the flask was put into another OKT3-solidifying flask to be cultivated. After 6 days, 250 ml of culture solution in the flask was mixed with 750 ml of LL-7 culture solution (made by Nikken Bio Medical Laboratory), and then, cultivated with a gas-penetrative cultivating bag in the atmosphere of 5% of carbonic acid gas at 37 ℃. Eight days after the

commencement of the experiment, 1 liter of culture solution was added and cultivated with two gas-penetrative cultivating bags. In the same manner, ten days after the commencement, the culture solution was cultivated with four bags, and then, twelve days after the commencement, it was cultivated with six bags. Fourteen days after the commencement, cells were gathered by a centrifugal separation process and suspended in about 250 ml of physiological saline containing 2% of human albumin, consequently to obtain a lymphocyte-suspended solution.

[EMBODIMENT 3] ADMINISTRATION OF LYMPHOCYTES

The lymphocyte-suspended solution obtained in Embodiment 2 was infused into the vein of the patient five times. For each infusion, about 1.2×10^{10} of lymphocytes were used. Consequently, the affected focus in the patient's eye due to the herpes simplex infections was cured. The result thereof revealed that the lymphocytes are efficacious against viral infections caused by EB viruses.

[EMBODIMENT 4] MEASUREMENT OF EB VIRUSES IN BLOOD

From the result of measuring, by a PCR measuring method, EB viruses in the blood of the patient to which the lymphocytes used in Embodiment 3 were applied, it was ascertained that the EB viruses in the blood are decreased with increasing the number of administration of the lymphocytes. As a result, the effect of the lymphocytes against the viral infections caused by EB viruses became evident.

[EMBODIMENT 5]

Different lymphocytes were prepared and cultivated in the same manner as that in Embodiment 2 noted above, except that the blood was drawn from the

vein of an HIV infected patient instead of drawing the blood from the vein of the patient contracting the Wiskott–Aldrich syndrome. Similarly to the lymphocytes of the patient contracting the Wiskott–Aldrich syndrome, the lymphocytes of the HIV infected patient could be proliferated, and fourteen days after the commencement of this experiment, the number of cells thereof reached 9×10^{9}.

INDUSTRIAL APPLICABILITY

According to this invention, the lymphocytes gathered from a patient are proliferated by being cultivated in the culture medium containing the anti–CD3 antibody and interleukin–2 made into a solid phase. Thus, there can be provided remedies or preventives for viral infections, which contain, as the main ingredient, the obtained autologous lymphocytes cultivated in a culture medium containing an anti–CD3 antibody and interleukin–2 made into a solid phase, and a method for preparing the remedies or preventives according to the invention. Furthermore, the therapeutic method by use of the remedies or preventives to be administered to a patient contracting the infections according to the invention can not only produce noticeable curative effects of treatment and prevention of viral infections, but also effectively prevent postoperative or post–transplantation viral infections.

Since the remedies and preventives according to this invention are effective agains the viral infections which are exceedingly difficult of treatment and prevention, they can also be used for treating or preventing bacilli, mycosis, protozoa and so on. Besides, the present invention makes it possible to sort out a specific type of lymphocytes from the remedies or preventives of the invention, add certain cells the remedies or preventives of the invention, and activate the remedies and preventives of the invention with cytokines such as

interleukin-12 for use in the treatment or prevention of the viral infections. By giving the remedies or preventives of the invention to a patient, the viral infections can be effectively treated or prevented by using jointly cytokines such as the interleukin-12 and various antiviral agents.

CLAIMS:

1. Remedies/preventives for treating or preventing viral infections, containing, as main ingredient, autologous lymphocytes proliferated by being cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase.

2. Remedies/preventives for treating or preventing viral infections according to claim 1, wherein said autologous lymphocytes are derived from a patient with immunodeficiency or immunosuppression.

3. A method for preparing remedies/preventives for treating or preventing viral infections, comprising cultivating autologous lymphocytes in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase, thereby to be proliferated.

4. A therapeutic method for preventing or treating viral infections, comprising administering, to a patient contracting the viral infections, remedies/preventives for viral infections, which remedies/preventives contain, as main ingredient, autologous lymphocytes proliferated by being cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase.

ABSTRACT

Remedies/preventives containing, as the main ingredient, autologous lymphocytes cultivated in a culture medium containing an anti-CD3 antibody and interleukin-2 made into a solid phase can improve the therapeutic or preventive effects on viral infections. Thus, the viral infections in patients with immunodeficiency or immunosuppression and postoperative or post-transplantation viral infections can be sufficiently prevented or treated by using the remedies/preventives.

DECLARATION AND POWER OF ATTORNEY FOR U.S. PATENT APPLICATION

(X) Original ( ) Supplemental ( ) Substitute ( ) PCT ( ) Design

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Title: REMEDIES/PREVENTIVES FOR VIRAL INFECTIONS, PROCESS FOR PREPARING THE SAME, AND METHOD FOR PREVENTING/TREATING VIRAL INFECTIONS

of which is described and claimed in:

( ) the attached specification, or

( ) the specification in the application Serial No. _____ filed _____;
and with amendments through _____ (if applicable), or

(X) the specification in International Application No. PCT/ JP97/02438, filed JULY 14, 1997, and as amended on _____ (if applicable).

I hereby state that I have reviewed and understand the content of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge my duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim priority benefits under Title 35, United States Code, §119 (and §172 if this application is for a Design) of any application(s) for patent or inventor's certificate listed below and have also identified below any application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NO.	DATE OF FILING	PRIORITY CLAIMED
JAPAN	HEI 8/204294	JULY 15, 1996	Yes

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disIcosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

APPLICATION SERIAL NO.	U.S. FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

And I hereby appoint John T. Miller, Reg. No. 21,120; Michael R. Davis, Reg. No. 25,134; Matthew M. Jacob, Reg. No. 25,154; Jeffrey Nolton, Reg. No. 25,408; Warren M. Cheek, Jr., Reg. No. 33,367; Nils E. Pedersen, Reg. No. 33,145 and Charles R. Watts, Reg. No. 33,142, who together constitute the firm of WENDEROTH, LIND & PONACK, L.L.P., attorneys to prosecute this application and to transact all business in the U.S. Patent and Trademark Office connected therewith.

I hereby authorize the U.S. attorneys named herein to accept and follow instructions from KOUICHI YOSHIMURA INTERNATIONAL PATENT OFFICE as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and myself. In the event of a change in the persons from whom instructions may be taken, the U.S. attorneys named herein will be so notified by me.

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I further declare that all statements made herein of my own knowledge are true, and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1st Inventor Teruaki Sekine Teruaki Sekine Date January 6, 1999

2nd Inventor _____ Date _____

3rd Inventor _____ Date _____

4th Inventor _____ Date _____

5th Inventor _____ Date _____

6th Inventor _____ Date _____

7th Inventor _____ Date _____

The above application may be more particularly identified as follows:

U.S. Application Serial No. _____ Filing Date _____

Applicant Reference Number _____ Atty Docket No. _____

Title of Invention _____